Glioblastoma multiforme is a highly malignant tumor of the central nervous system (CNS) with aggressive histological features and an extraordinarily poor clinical outcome. However, the extraneural metastasis of glioblastoma multiforme is distinctly unusual. Several explanations have been postulated to account for this observation. One of the important considerations is the absence of cerebral lymphatic vessels. Another is the unique vasculature of the CNS. The intracranial sinuses are enclosed in a dense dural membrane, thus making penetration difficult. The cerebral veins, on the other hand, are thin walled. Some researchers have suggested that these vessels would likely collapse from compression before allowing penetration by an expanding tumor. Another reason for the rarity of metastases from glioblastoma multiforme may be because of the poor prognosis, which includes very short patient survival times. In addition, some authors have theorized that the glial tumor cells require a special metabolic environment to survive, which is provided only in the CNS.

We found 247 well-documented cases of glioblastoma multiforme with extraneural metastasis in previous reports and recent reviews. However, 237 of these instances occurred after neurosurgical procedures, including craniotomy or ventriculoperitoneal (VP) shunt placement. Therefore, it appears that the most frequent single factor in the development of extraneural metastasis is the direct access of glioblastoma multiforme cells to the extrameningeal tissues. After the surgical procedure, the lymphatics outside the nervous system are exposed to the tumor. In addition, the intracerebral vasculature will certainly be open and at risk for tumor cell seeding. Finally, an unusual but documented mode of spread is through VP shunts.

Based on these observations, we postulated that in the human glioblastoma multiforme xenotransplanted to an extraneural site, for example, subcutaneous tissue with a rich lymphatic and venous supply, the metastatic potential would be different from that in the CNS.
would be more fully expressed. The published data on xenografts of human glioblastoma multiforme in nude mice shows that many tumors do grow progressively and that some retain the histological characteristics of the original tumors.\(^6\) However, metastasis was not recorded in these studies. This absence of distant metastasis may be due, in part, to the residual immune reactivity of nude mice against human xenografts.\(^6,26-28,34\)

The severe combined immunodeficient (SCID) mouse, which was first described by Bosma, et al.,\(^3\) is more severely immunocompromised than the nude mouse and might be a better model in which to examine the metastatic potential of glioblastoma multiforme. The SCID mouse lacks both functional B and T cells because of non-functional rearrangements of immunoglobulin (Ig) and T-cell receptor genes.\(^3\) Although no metastatic data on human glioblastoma multiforme xenografts in SCID mice have been reported, several recent reports have demonstrated that SCID mice accepted xenografting of human tumor and developed spontaneous metastasis in human nonglioblastoma multiforme xenografts at a higher frequency than nude mice.\(^7,10,30,32\)

In the present study, we examined the ability of SCID mice to support the growth of five human glioblastoma multiforme xenografts and the expression of their metastatic behavior after amputation of the leg bearing 10-mm tumors. Concurrently, we compared these results with those of nine other human tumor xenografts.

### Materials and Methods

#### Animal Model

Seven- to 8-week-old male SCID mice from our defined flora- and specific pathogen-free animal colony\(^\)* were used. The original SCID mice in our colony were generously provided by Dr. Gloria Ku (Merck Co., Inc., Rahway, NJ) in 1989. The IgG production was measured and found to be nonsignificant. During the experimental period, the mice used in this study were maintained in microisola- tors, fed with sterile laboratory pellets, and given acidified sterile water ad libitum. The guidelines for the humane treatment of ani- mals used in this study were approved by the Subcommittee on Research Animal Care, Office of Laboratory Animal Resources at the Massachusetts General Hospital.

#### Tumor Cell Lines

A total of 14 human tumor xenografts were used in this study. They included 5 glioblastoma multiforme xenografts: U87, MMC1, HGL13, HGL16B, and HGL21; four squamous cell carcinomas: FaDu, SCC21, JSQ3, and SQ20B; two soft-tissue sarcomas: HSTS26T and HSTS11; and a neuroblastoma SKNMC, a colon carcinoma HCT15, and a breast carcinoma MDA-MB-231, respec- tively. All of the xenografts came from well-established cell lines, which were tumorigenic in immunodeficient nude and SCID mice, and have been used as solid tumor xenografts previously passaged in vivo. The cell lines HGL13, HGL16B, HGL21, HSTS11, and SCC21 were derived from patients at the Massachusetts General Hospital and established in our laboratory. The cell lines HSTS26T, SQ20B, and JSQ3 were established in the laboratory of Dr. Little (Harvard School of Public Health, Cambridge, MA), and kindly provided by W. Dahlberg. The U87, HCT15, SKNMC, MDA-MB-231, and FaDu cell lines were obtained from the American Type Culture Collection (Rockville, MD). The MMMC1 cell line was derived from a patient at the Montefiore Medical Center, New York, New York, and was kindly provided by Dr. Kornblith.

#### Tumor Transplantation

All the xenografted tumors were obtained from the second to eighth generation source in our laboratory and were excised, cleared of necrotic tissue, cut into small chunks, and transplanted subcu- taneously into the right hindleg of the experimental mice. Twenty-one to 25 SCID mice were used for each tumor type, for a total of 340 animals.

### Growth Characteristics

The parameters of “take” rate, latent period (the number of days required for the development of palpable subcutaneous tumors post- transplantation), volume doubling time (VDT, the number of days required for a tumor to double its volume), and growth time (GT, the number of days required for the tumors to grow to the volume of 500 ± 50 mm\(^3\)) were used to assess the growth characteristics of these xenografts. Two perpendiculardiameters of the tumors were measured twice a week with calipers until the tumors were surgically treated. Tumor volume (V) was calculated as \(V = \frac{a \times b}{2}\), where \(a\) and \(b\) are the long and short axes, respectively. The volume of the individual tumor was also used to calculate the VDT according to the following formula: \(VDT = \ln(2) \times \frac{\ln(V)}{\ln(V/V_0)}\), where \(V_0 = 500 ± 50\ mm^3\) is determined in all the tumor types, \(V\) is the primary volume (approximately 18 mm\(^3\)) for each tumor at time \(T_0\) (which corresponds to the beginning of tumor take). This \(T_0\) occurred between 5 and 40 days for the 14 different tumor lines.

#### Surgical Procedures

When the subcutaneously transplanted tumors reached the size of 10 × 10 mm (500 ± 50 mm\(^3\)), the mice were anesthetized with sodium pentobarbital (0.05 mg/g body weight) given by intraperi- toneal injection. The tumor-bearing leg was prepared with 70% alcohol, the blood flow was stopped by means of a clamp attached at midthigh for 3 to 5 minutes, and then the leg was amputated. The resulting wound was closed with a 9-mm wound autoclip (Clay Adams, Division of Becton Dickinson and Co., Parsippany, NJ), and the wound clips were removed 2 to 3 weeks later.

#### Metastasis Determination

Following amputation, the mice were examined at least three times a week for up to 5 months after tumor transplantation. Post-mortem examination for metastasis to regional nodes and the abdominal or thoracic viscera was performed on all the mice. Both functional B and T cells because of non-functional rearrangements of immunoglobulin (Ig) and T-cell receptor genes.

### Results

#### Growth of Glioblastoma Multiforme Versus Other Histological Types of Xenografts

Table 1 gives the transplant take results and the mean growth and metastasis of human glioblastoma in SCID mice.
The xenotransplant take rate, latent period, the VDT, and GT were analyzed according to the histological types of tumor (glioblastoma multiforme vs. squamous cell carcinoma and adenocarcinoma; and vs. soft-tissue sarcoma). As shown in Table 1 and Fig. 3, glioblastoma multiforme exhibited longer latent periods, VDT, GT, and a lower take rate in comparison with the other tumors tested. However, the difference did not achieve statistical significance (Mann–Whitney U-test, $p > 0.05$), and there was a broad overlap of values for these parameters.

**Local Recurrence and Spontaneous Metastases**

Table 2 shows the incidence of spontaneous metastases, as well as the site of metastasis in each tumor xenografted type. Table 3 gives the local recurrence results and their relationship to metastases in the five human glioblastoma multiforme xenografts compared with the other nine tumors in SCID mice following amputation.

There was considerable variability in the local recurrence and spontaneous metastasis rate among the five human glioblastoma multiforme xenografts in SCID mice. Two glioblastoma multiforme xenografts (HGL16B and HGL21) showed higher rates of local recurrence (77.8% and 68.2%) and metastasis to lymph node (88.8% and 54.5%) than distant metastatic rates (55.6% and 13.6%). For the other three glioblastoma multiforme xenografts (U87, MMC1 and HGL13) the incidence of metastasis was 12%, 4.2%, and 0%, and 0%, 0%, and 5.3% for local recurrence, respectively.
Growth and metastasis of human glioblastoma in SCID mice

Fig. 2. Graphs showing growth curves of two soft-tissue sarcomas (HSTS26T and HSTS11) and three squamous cell carcinoma xenografts (FaDu, SQ20B, and SCC21) transplanted subcutaneously in severe combined immunodeficient mice. Both the latent period and the growth pattern are shown. Each xenograft type consisted of 20 to 25 tumors.

TABLE 2
Incidence and sites of metastases of human glioblastomas multiforme compared with other histological types of human tumor xenografts in SCID mice after surgical treatment*

<table>
<thead>
<tr>
<th>Designation</th>
<th>Incidence of Metastases (%)</th>
<th>Lung</th>
<th>Liver</th>
<th>Lymph Node</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>glioblastoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGL13</td>
<td>0/19 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HGL16B</td>
<td>16/18 (88.9)</td>
<td>55.6</td>
<td>5.6</td>
<td>88.9</td>
<td>0</td>
</tr>
<tr>
<td>HGL21</td>
<td>12/22 (54.5)</td>
<td>13.6</td>
<td>0</td>
<td>40.9</td>
<td>0</td>
</tr>
<tr>
<td>MMC1</td>
<td>1/24 (4.2)</td>
<td>4.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>U87</td>
<td>3/25 (12)</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>soft-tissue sarcoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSTS11</td>
<td>3/25 (12)</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HSTS26T</td>
<td>2/23 (100)</td>
<td>100</td>
<td>0</td>
<td>91.7</td>
<td>30.8</td>
</tr>
<tr>
<td>squamous cell carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC21</td>
<td>7/20 (35)</td>
<td>15</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>SQ20B</td>
<td>0/25 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>JSQ3</td>
<td>2/23 (8.7)</td>
<td>4.3</td>
<td>0</td>
<td>4.3</td>
<td>0</td>
</tr>
<tr>
<td>FaDu</td>
<td>25/25 (100)</td>
<td>96</td>
<td>96</td>
<td>56</td>
<td>64</td>
</tr>
<tr>
<td>adenocarcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT15</td>
<td>1/25 (4)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>21/21 (100)</td>
<td>100</td>
<td>14.3</td>
<td>100</td>
<td>23.8</td>
</tr>
<tr>
<td>neuroblastoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKNMC</td>
<td>1/18 (5.6)</td>
<td>5.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* SCID = severe combined immunodeficient.

TABLE 3
Local recurrence and metastases in human glioblastomas multiforme compared with other histological types of human tumor xenografts in SCID mice after surgical treatment*

<table>
<thead>
<tr>
<th>Designation</th>
<th>Rate of Local Recurrence (%)</th>
<th>No. Metastases/No. Local Recurrences</th>
<th>No. Metastases/No. Local Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>glioblastoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGL13</td>
<td>1/19 (5.3)</td>
<td>0/1</td>
<td>0/18</td>
</tr>
<tr>
<td>HGL16B</td>
<td>14/18 (77.8)</td>
<td>13/14</td>
<td>3/4</td>
</tr>
<tr>
<td>HGL21</td>
<td>15/22 (68.2)</td>
<td>10/15</td>
<td>2/7</td>
</tr>
<tr>
<td>MMC1</td>
<td>0/24 (0)</td>
<td>0/0</td>
<td>1/24</td>
</tr>
<tr>
<td>U87</td>
<td>0/25 (0)</td>
<td>0/0</td>
<td>3/25</td>
</tr>
<tr>
<td>soft-tissue sarcoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSTS11</td>
<td>0/25 (0)</td>
<td>0/0</td>
<td>3/25</td>
</tr>
<tr>
<td>HSTS26T</td>
<td>9/23 (39.1)</td>
<td>9/9</td>
<td>14/14</td>
</tr>
<tr>
<td>squamous cell carcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC21</td>
<td>1/20 (5.0)</td>
<td>1/1</td>
<td>6/19</td>
</tr>
<tr>
<td>SQ20B</td>
<td>11/25 (44)</td>
<td>0/11</td>
<td>0/14</td>
</tr>
<tr>
<td>JSQ3</td>
<td>3/23 (13)</td>
<td>1/3</td>
<td>1/20</td>
</tr>
<tr>
<td>FaDu</td>
<td>0/25 (0)</td>
<td>0/0</td>
<td>25/25</td>
</tr>
<tr>
<td>adenocarcinoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT15</td>
<td>1/25 (4.0)</td>
<td>0/1</td>
<td>1/24</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>9/21 (42.9)</td>
<td>9/9</td>
<td>12/12</td>
</tr>
<tr>
<td>neuroblastoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKNMC</td>
<td>0/18 (0)</td>
<td>0/0</td>
<td>1/18</td>
</tr>
</tbody>
</table>

* SCID = severe combined immunodeficient.
The metastatic incidence of the nine other histological types of human tumor xenografts varied widely: 100% for HSTS26T, FaDu, and MDA-MB-231; less than 25% for SCC21; and nonmetastasis for SKNMC. Of the three highly metastatic xenografts, FaDu produced distant metastases to lungs (96%) and livers (96%); the HSTS26T and MDA-MB-231 metastasized to lungs in all mice and to regional lymph nodes in 91.7% and 100% of the animals, respectively. However, no significant difference was found in the incidence of metastasis between the five glioblastomas multiforme and the nine other histological xenografts in our studies (Mann–Whitney U-test, p < 0.05).

As shown in Table 3, there was a significantly higher metastatic incidence in the mice with locally recurrent tumors (67%) compared with the mice with locally controlled tumors (29%; p < 0.005). However, no correlation was found between the local recurrence rate and the metastatic incidence in these xenografts (r² = 0.278). Also, no correlation was found between the VDT, GT, and the metastatic incidence in these xenografts (r² = 0.08 and 0.07, respectively).

In the xenografts with highly metastatic potential, the time to maximum expression of spontaneous metastasis was between 30 and 90 days after surgical treatment. However, for the poorly metastatic xenografts, allowing mice to live for approximately 150 days after tumor transplantation did not increase the metastatic incidence.

**Examination of Pathomorphology**

Macroscopic examination of these subcutaneously growing tumors at the site of transplantation showed round, oval, or lobulated masses. Most were well localized and grew in the subcutaneous space between the skin and the muscles of the leg. However, at diameters of 8 to 10 mm, central necrosis and surface ulceration were common in xenografts of FaDu, SCC21, JSQ3, MDA-MB-231, HGL21, and HGL16B.

Although the subcutaneous xenografts were well circumscribed, on microscopic examination we found that most were not encapsulated, and nests of tumor cells were observed invading the surrounding connective tissue, fat, muscle, and the covering skin.

Most of these xenografts retained the individual histological features of their original tumor types. The five human glioblastoma multiforme xenografts were morphologically unique and reproduced their own histological features in the host. For example, high cellularity was found in HGL16B tumors (Fig. 4A), which were composed of many cells with round or oval nuclei and ill-defined cytoplasmic outlines and processes, and were punctuated by numerous large giant cells with darkly stained nuclei. Mitotic figures were easily found in most fields. Many thin-walled blood vessels were present in the tumor stroma. Broad swirling bands of spindle-shaped cells with many mitotic figures, and punctuated by bizarre giant cells, were seen in the HGL21 line (Fig. 4B). Focal necrosis with pseudopalisading could be seen in some...
areas, but was not present in the field. The U87 line (Fig. 4C) showed highly pleomorphic tumor cells with irregular nuclei and abundant cytoplasm. Mitoses and vascular stroma were consistent features.

Metastatic foci in the lungs (Fig. 4D and E), liver, lymph nodes, and kidneys were identified macroscopically. Most of the metastatic foci were presented as white or gray nodules on the surface of the lungs or liver. Metastatic foci in lymph nodes caused their volume to increase markedly, with irregular shapes resulting. Metastatic formations were confirmed histologically in three to 10 organs (lungs or liver) of tumor-bearing hosts from each type of xenograft (Fig. 5A and B). A close morphological resemblance between the subcutaneous xenografts and their metastatic colonies in distant organs was found in all the examined cases.

Discussion
Glioblastoma multiforme is the most poorly differentiated neoplasm of glial origin and has an exceptionally poor prognosis. The fatal outcome after surgery and/or

![Fig. 4. A–C: Photomicrographs of three glioblastomas multiforme xenografts: HGL16B (A), HGL21 (B), and U87 (C) transplanted subcutaneously into severe combined immunodeficient (SCID) mice (arrow, vascular stroma). H & E, original magnification × 200. D and E: Photographs of lung metastases in SCID mice bearing HGL21 (D) and U87 (E) xenografts. Metastatic foci were presented as white or gray nodules on the surface of the lungs (arrows).](image1)

![Fig. 5. Photomicrographs showing lung (A) and liver (B) metastases in severe combined immunodeficient mice bearing HGL16B glioblastoma multiforme xenografts. H & E, original magnification × 200 (A); × 100 (B).](image2)
A number of human glioblastomas multiforme have been successfully xenografted in genetically immunodeficient nude mice.\textsuperscript{1,4,5,21,25} Xenografted tumor models allow researchers to test diverse therapeutic agents against specific types.\textsuperscript{1,2,5,25,29} The proportion of human tumor that can be successfully transplanted or progressively grown in nude mice varies widely; for glioblastoma multiforme the figure is 26.7\% to 100\%,\textsuperscript{1,4,5,21,25} For established human xenografts of nonneural origin in nude mice, spontaneous metastases were found only at low to modest rates, even when they were derived from highly metastatic tumors.\textsuperscript{3,6,8,13,14,17,18,20,22,33} No metastatic data for human glioblastoma multiforme xenografts has been reported. The growth and metastasis of human tumors in nude mice might be limited by the animal’s residual immunity.\textsuperscript{27,28} In fact, several reports have demonstrated that the use of immunologically immature young or immunosuppressed nude mice could increase the take rate and metastatic ability of human xenografts.\textsuperscript{11,15,27,34} Thus, a mouse more genetically immunodeficient, such as the SCID mouse, may serve as a better model for the study of the malignant biological behavior of human glioblastoma multiforme xenografts.

In the present study, five human glioblastoma multiforme xenografts successfully grew after subcutaneous transplantation into SCID mice (72\%–100\% take rate). Three cell lines expressed a local recurrence, and four developed spontaneous distant metastases. Although the metastatic activity in these five glioblastoma multiforme xenografts was variable (from 0\%–88.9\%), this is the first report to demonstrate that human glioblastoma multiforme can express spontaneous metastases in SCID mice following subcutaneous transplantation. No significant difference was found in the incidence of metastases between the five glioblastoma multiforme xenografts and the nine other histological tumors (p > 0.05). This study also revealed the high local/regional recurrence rates (77.8\% and 68.2\% local recurrences, 88.9\% and 54.5\% local lymph node metastases) following midthigh amputation of 10-mm tumors in two glioblastoma multiforme xenografts, in comparison with other histological types in this animal model. Although there was a higher metastatic incidence in the mice with locally recurrent tumors, compared with mice with locally controlled tumors, no correlation was found between the local recurrence rate and the metastatic incidence in this study.

Conclusions

In conclusion, these results demonstrate that the metastatic behavior of the human glioblastoma multiforme xenografts does not differ significantly from that seen in a variety of other histological xenografts when evaluated at the same transplantation site in the SCID model. These results are consistent with the hypothesis that the site of glioblastoma multiforme growth influences the extraneuronal metastatic spread of this disease and lead us to suggest that the clinical rarity of distant metastasis is not a fundamental property of these cells. This study also shows that the SCID mouse is an attractive model for further biological and preclinical studies of human glioblastoma multiforme.

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